Supporting Information for:

1-Oxo-5-hydroxytryptamine: A Surprisingly Potent Agonist of the 5-HT_3 (Serotonin) Receptor

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Chemistry—General.

All reactions were performed under Argon using solvents that were dried and purified according to the method of Grubbs.[1] All flasks and vials were oven dried at 122°C and cooled in a desiccator box containing anhydrous calcium sulfate. Commercial reagents were purified according to the methods compiled by Armengo and Perrin.[2] Hydrogen chloride gas was generated in situ by dropwise addition of concentrated HCl into stirred concentrated H_2SO_4. Reactions were monitored by thin-layer chromatography on Merck Siligel 60-F_{254}. Compounds were visualized with UV lamp (254 nm) and stained with ceric ammonium molybdate solution. Column chromatography was carried out in accordance with the methods of Still[3] using EMD-Merck silica gel 60, 230-400 mesh ASTM or Aldrich Brockmann I alumina, 150 mesh (for acid sensitive compounds such as pyrroline). ^{1}H and ^{13}C NMR spectra were acquired on a Varian Mercury 300 MHz spectrometer. High-resolution mass spectrometry was performed on a JEOL JMS-600H HRMS using an Electrospray Ion Source.

Chemistry—Synthesis.

(**6**). Boron trifluoride-diethyl etherate (0.6 mL, 5 mmol) was added to a mixture of acetonitrile (10 mL) and dichloromethane (30 mL) in a 100 mL roundbottomed flask and cooled to -78 °C. A solution of *p*-benzoquinone (540 mg, 5 mmol) and 2,3-dihydrofuran (0.6 mL, 7.5 mmol) in acetonitrile (10 ml) was then added to the stirred BF_3 solution at -78 °C by syringe pump over 2 h. After 5 total hours, additional 2,3-dihydrofuran (0.6 mL, 7.5 mmol) was added dropwise over 10 minutes. After 8 total hours, The reaction temperature was allowed to slowly rise to room temperature over the following hour, at which point methanol (10 mL) and 1 N HCl (10 mL) were added. The product was extracted with 4 x 30 mL dichloromethane, washed with 1 x 30 mL brine, dried on magnesium sulfate, and reduced *iv*. The residue was filtered through a silica plug with ethyl acetate, then purified by flash chromatography on silica (25% EtOAc in hexanes). The product fraction could be purified further by recrystallization from 3 mL toluene to yield flaky, white crystals; 160 mg (18%) **6**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.59-6.70 (m, 3H), 6.27 (d, 1H, J = 6 Hz), 4.66 (s, 1H, OH), 4.05 (t, 1H, J = 8 Hz), 3.95 (t, 1H, J = 7 Hz), 3.58-3.64 (m, 1H), 2.22-2.29 (m, 1H), 2.03 (dd, 1H, J = 12, 4.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.6, 150.5, 128.9, 115.5, 112.1, 111.3, 109.6, 67.5, 47.2, 33.6; HRMS (EI^+) calculated for [$C_{10}H_{10}O_3$] ([M]$^+$) 178.0630, found 178.0638. The characterization data matched reported values for this compound synthesized using the Corey oxazaborolidine catalyst.[4]

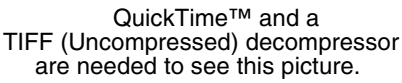
(**8**). **6** (89 mg, 0.50 mmol) was measured into a 2-dram vial, and acetonitrile (5 mL) was added to dissolve it. Boron trifluoride-diethyl etherate (140 μL, 1.1 mmol) was then added. The vial was then shaken at 37°C for 22 h. Aqueous HCl (4 mL, 1 N) was then added, and the product was extracted with 5 x 8 mL dichloromethane, dried on sodium sulfate, and reduced *iv*. The residue was then purified by flash chromatography on silica (50% EtOAc in hexanes) to yield 74.1 mg (83%) **8**. ^{1}H NMR (300 MHz, acetone-d_6) δ 8.03 (s, 1H), 7.58 (s, 1H), 7.27 (d, 1H, J = 9 Hz), 7.01 (d, 1H, J = 3 Hz), 6.81 (dd, 1H, J = 9, 3 Hz), 3.99 (s, 1H), 3.82 (t, 2H, J = 6 Hz), 2.82 (t, 2H, J = 6 Hz); ^{13}C NMR (75 MHz, acetone-d_6) δ 153.9, 150.0, 143.8, 129.8, 118.0, 113.5, 112.0, 104.9, 61.6, 27.5; HRMS (EI^+) calculated for [$C_{10}H_{10}O_3$] ([M]$^+$) 178.0630, found 178.0628.

(**10**). **8** (193 mg, 1.1 mmol), triphenylphosphine (341 mg, 1.3 mmol), and N-Boc-*o*-nitrobenzenesulfonamide[5] (393 mg, 1.3 mmol) were measured into a 2-dram vial. THF (5 mL) was added. Diisopropyl azodicarboxylate (256 μL, 1.3 mmol) was added to the stirred solution by a syringe over a 10 minute period. After an additional 20 minutes stirring at room temperature, the reaction was reduced *iv*, and the residue was triturated with toluene (5 mL) to give 372.7 mg of a white solid. Additional product could be recovered by flash chromatography of the filtrate on silica (30% EtOAc in hexanes), followed by trituration of the product fraction to give a total yield of 483.0 mg (96 %) **10**. ^{1}H NMR. (300 MHz, acetone-d_6) δ 8.28-8.31 (m, 1H), 7.95-8.02 (m, 3H), 7.70 (s, 1H), 7.32 (d, 1H, *J* = 9 Hz), 7.14 (d, 1H, *J* = 2 Hz), 6.86 (dd, 1H, *J* = 9, 2 Hz), 4.03 (t, 2H, 7 Hz), 3.08 (t, 2H, 7 Hz), 1.27 (s, 9H); ^{13}C NMR (75 MHz, acetone-d_6) δ 153.7, 150.4, 150.0, 143.6, 135.3, 133.0, 132.6, 132.5, 128.9, 124.9, 116.6, 113.4, 111.7, 104.5, 85.0, 47.2, 27.1, 21.6; HRMS (EI$^+$) calculated for [$C_{21}H_{22}N_2O_8S$] ([M]$^+$) 462.1097, found 462.1100.

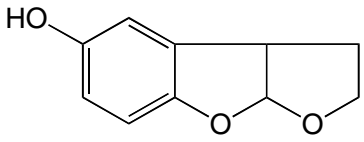
1-oxa-5-hydroxytryptamine hydrochloride (**2**). **10** (445 mg, 0.96 mmol) was measured into a 25 mL round-bottomed flask. DMF (9.6 mL), followed by lithium hydroxide monohydrate (162 mg, 3.85 mmol) and mercaptoacetic acid (134 μL, 1.93 mmol) were then added. After 6 h room temperature stirring, the reaction was poured onto 60 mL aqueous sodium bicarbonate, and the product was extracted with 5 x 20mL EtOAc, washed with 1 x 20 mL brine, reduced *iv*, and filtered through a silica plug (30% EtOAc in hexanes) to yield 247 mg, which was transferred into a 50 mL 3-neck flask appended with an HCl purge. Chloroform (20 mL) and methanol (2 mL) were added, and HCl was purged through the stirring solution for 30 minutes. The reaction was then reduced *iv*, and the residue was redissolved in acetone (5 mL). This acetone solution was then added dropwise to a stirring beaker of ether (20 mL); the resultant white crystals were then isolated by filtration to yield 130 mg (64%, 2 steps) 1-oxa-5-hydroxytryptamine hydrochloride. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.31 (s, 1H, OH), 8.13 (s, 3H, NH), 7.78 (s, 1H), 7.34 (d, 1H, *J* = 8 Hz), 6.95 (d, 1H, *J* = 2 Hz), 6.78 (dd, 1H, *J* = 9, 2 Hz), 3.06 (t, 2H, *J* = 8 Hz), 2.91 (t, 2H, *J* = 8 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 154.0, 149.4, 144.1, 128.7, 116.1, 113.9, 112.3, 104.7, 38.7, 22.0; HRMS (EI$^+$) calculated for [$C_{10}H_{11}NO_2$] ([M]$^+$) 177.0790, found 177.0794.

Molecular Biology and Electrophysiology

The measurement of EC_{50} and efficacy values for 5-HT, 1-OT and 5-FT was carried out as previously described.[6] Briefly, the 5-HT_3 receptor was expressed in *Xenopus laevis* oocytes by manual injection of the corresponding mRNA. Following 1-3 days incubation at 18 °C, transmembrane current in response to drug application was measured using a two-electrode whole-cell voltage clamp. From these data a dose-response curve was created for each drug and was fit to the Hill equation.



NMR Data



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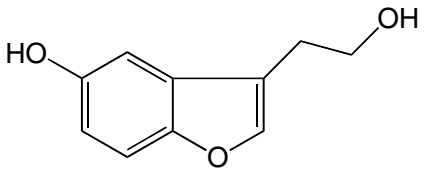

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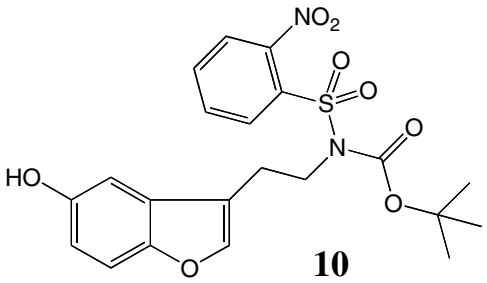

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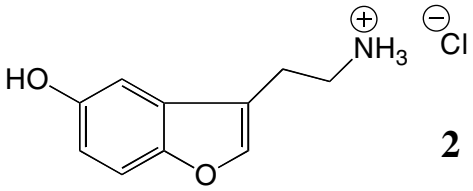

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